APA896Ra61 100μg
Active Cystatin C (Cys-C)
Organism Species: *Rattus norvegicus (Rat)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Gly21~Ala140
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 9.2
Predicted Molecular Mass: 14.9kDa
Accurate Molecular Mass: 16kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the

protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

GTSRPPPRLL GAPQEADASE EGVQRALDFA
VSEYNKGSND AYHSRAIQVV RARKQLVAGI NYYLDVEMGR TTCTKSQTNL
TNCPFHDQPH LMRKALCSFQ IYSVPWKGTH TLTKSSCKNA

[ACTIVITY]

Cystatin C is a member of family 2 of the Cystatin superfamily. It is involved in processes such as tumor invasion and metastasis, inflammation and some neurological diseases. It inhibits many cysteine proteases such as papain and cathepsins B, H, K, L and S. It is ubiquitous in human tissues and body fluids. A point mutation in the gene coding for the 120 amino acid mature Cystatin C causes a hereditary form of amyloid angiopathy in which the protein variant (Leu68 to Gln) is deposited in the cerebral arteries, leading to fatal cerebral hemorrhage. Cystatin C may have additional clinical applications. For example, it is a good marker for glomerular filtration rate. The activity of recombinant rat Cystatin C was measured by its ability to inhibit papain cleavage of a fluorogenic peptide substrate Z-FR-AMC in the assay buffer 50 mM Tris, pH 7.0. Papain was diluted to 500 ug/ml in activation buffer 50 mM Tris, 5 mM DTT, pH 7.0 and incubated at room temperature for 15 minutes. The activated papain was diluted to 100 ug/ml in the assay buffer and 20 ul different concentrations of recombinant rat Cystatin C (MW: 14.93 KD) was incubated with 20 ul 100 ug/ml papain at 37 ℃ for 10 minutes. Loading 50 µL of the incubated mixtures which were diluted five-fold in assay buffer into empty wells of a plate, and start the reaction by adding 50 µL of 200 µM substrate. Include a substrate blank containing 50 µL of assay buffer and 50 µL of 200 µM substrate. Then read at excitiation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes. The result was shown in Figure 1 and it was obvious that recombinant rat Cystatin C significantly

decreased papain activity. The inhibition IC50 was <80 nM.

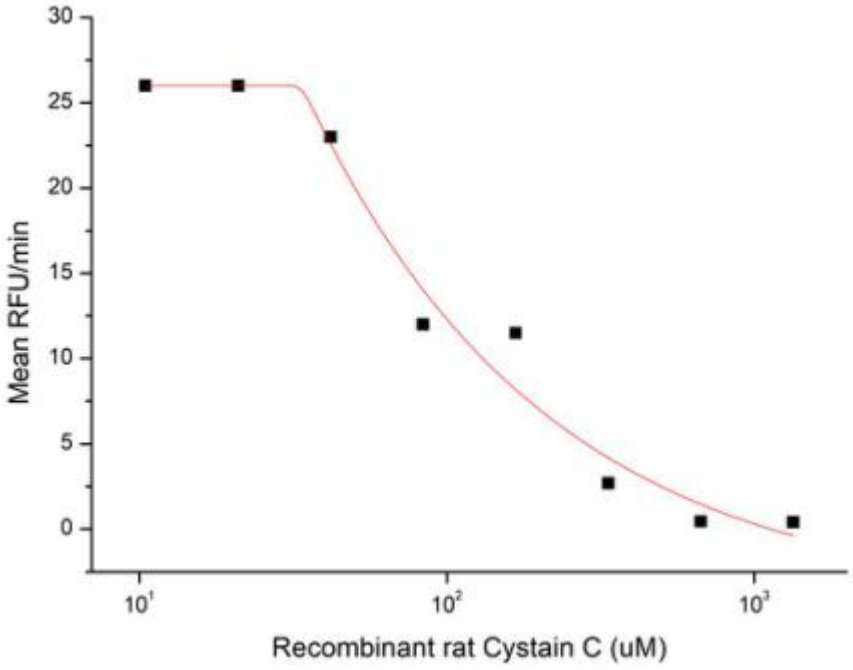

Figure 1. Inhibition of papain activity by recombinant rat Cys-C

[IDENTIFICATION]

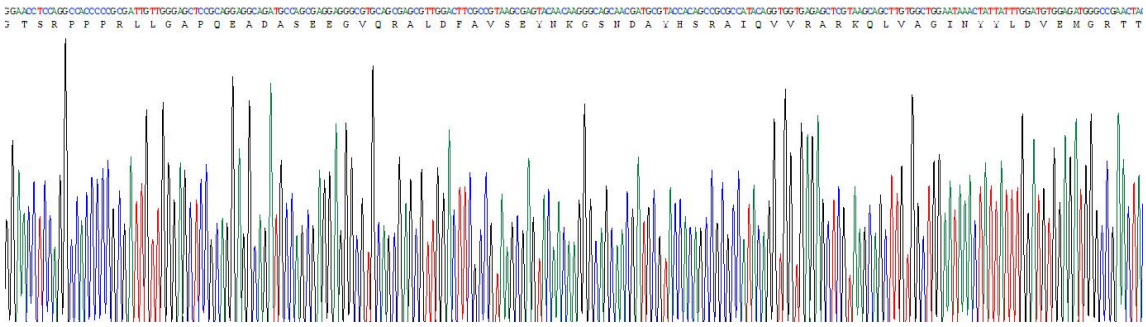

Figure 2. Gene Sequencing (extract)

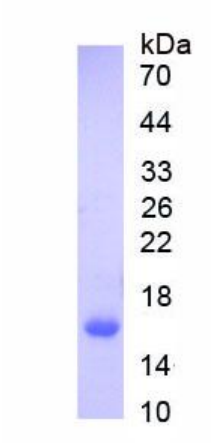

Figure 3. SDS-PAGE

Sample: Active recombinant Cys-C, Rat

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.